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Caspase-11: The Noncanonical Guardian of Cytosolic Sanctity

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Caspase-11 controls a noncanonical inflammasome that responds to Gram-negative bacteria and certain pore-forming toxins. Aachoui et al. (2013) and Case et al. (2013) identify a role for caspase-11 in rapid responses to bacterial pathogens that access the cytosol via vacuolar membrane disruption or the activity of specialized secretion systems.

Cells of the innate immune system detect microbial infection through membrane-bound and cytosolic pattern recognition receptors that enable discrimination between self and nonself and between pathogenic and nonpathogenic bacteria. Activation of caspase-1 plays a key role in antimicrobial immunity, as caspase-1 mediates a lytic cell death termed pyroptosis that is associated with the secretion of caspase-1-dependent proinflammatory cytokines (Bergsbaken et al., 2009). In particular, caspase-1 is required for the processing and secretion of interleukin-1β (IL-1β) and IL-18. Mechanisms governing IL-1α release are less clear, as IL-1α is not directly processed by caspase-1, yet its release is still associated with caspase-1 activation. Caspase-1 activation occurs through its recruitment to Nod-like receptor (NLR)-containing complexes termed inflammasomes, which have been proposed to be guardians of "cytosolic sanctity" against the presence of microbial products (Lamkanfi and Dixit, 2009). NLRs mediate inflammasome assembly in response to particular infection- or stress-associated stimuli and recruit caspase-1 either through direct homotypic interactions between caspase recruitment (CARD) domains or through an intermediate adaptor protein known as ASC, which contains a CARD domain and a Pyrin domain. The Pyrin domain of ASC allows it to interact both with the N-terminal Pyrin domain of certain NLRs such as NLRP3 and the CARD of caspase-1.

Early work by the group of Junying Yuan demonstrated a critical role for caspase-11 in caspase-1 activation and also demonstrated that caspase-11 was essential for the induction of endotoxic shock in vivo (Wang et al., 1998). More recently, Kayagaki et al. reported that all *Casp1*$^{-/-}$ mice also lack caspase-11, due to the generation of the *Casp1*$^{-/-}$ line in the 129 mouse strain background, which express a mis-spliced and truncated version of the *Casp11* messenger RNA (Kayagaki et al., 2011). This raised the question of whether phenotypes initially attributed to caspase-1 were in fact due to the absence of caspase-11 in these mice. Intriguingly, Kayagaki et al. found that Gram-negative bacteria and certain pore-forming toxins induce a caspase-11-dependent noncanonical inflammasome that contributes to NLRP3-dependent IL-1β release and also triggers NLRP3- and caspase-1-independent cell death and IL-1α release.

The mechanisms underlying caspase-11-dependent cell death and the precise contributions of caspase-11 to caspase-1-dependent and -independent inflammatory processes remain poorly understood. One feature of caspase-11 activation in response to Gram-negative bacteria appears to be the requirement for LPS-mediated signaling (Broz et al., 2012; Gurung et al., 2012; Rathinam et al., 2012). Induction of type I interferon (IFN) and subsequent type I IFN receptor (IFNAR) signaling through TLR4, which recognizes LPS, and the adaptor protein TRIF upregulates procaspase-11 expression, and this upregulation is required for caspase-11 processing and activation. Nevertheless, basal levels of procaspase-11 are still present in TRIF-deficient cells, suggesting that additional signals play an important role in caspase-11 activation (Broz et al., 2012). Furthermore, while type I IFN signaling is sufficient to induce caspase-11 upregulation and processing (Rathinam et al., 2012), LPS treatment or type I IFN treatment alone does not cause caspase-11-dependent cell death (Broz et al., 2012), further implicating additional signals in caspase-11 activation.

Two new studies now shed light on additional mechanisms of caspase-11 activation and demonstrate that caspase-11 responds to bacterial pathogens that access the host cell cytosol, either via specialized secretion systems that translocate bacterial products into the host cytosol or through direct entry of bacteria into the cytosol after vacuolar lysis (Aachoui et al., 2013; Case et al., 2013). Case et al. analyzed the mechanisms underlying inflammasome activation by *Legionella pneumophila*, which establishes an intracellular vacuolar niche using a *dot/icm*-encoded type IV secretion system (T4SS) to translocate bacterial virulence factors into the host cytosol. Previous studies had demonstrated that *Legionella* with a functional T4SS trigger ASC-dependent IL-1β secretion that is independent of the flagellin/NAIP5/NLRC4 inflammasome, but the host factors involved were unknown. Through the elegant use of bacterial mutants in combination with mice lacking various inflammasome components or harboring individual deletions in caspase-1 or caspase-11, Case et al. now demonstrate that this ASC-dependent pathway requires caspase-11. They also show that caspase-11 contributes to a second,

ASC-independent pathway that results in cell death. Intriguingly, Case et al. show that *Legionella* triggers rapid caspase-11-dependent cell death in LPS-primed cells within 4 hr of infection, in contrast to other Gram-negative bacterial infections, for which the caspase-11-dependent response generally requires 16–20 hr. This more rapid caspase-11-dependent cell death and cytokine secretion requires *Legionella* with a functional T4SS (Figure 1). Furthermore, while NLRP3 and ASC were important for processing of caspase-1 and IL-1β in response to flagellin-deficient (Δ*flaA*) *Legionella*, NLRP3, ASC, and caspase-1 were dispensable for caspase-11-dependent cell death and IL-1α release. Additionally, they find that although IFNAR signaling is not required for caspase-11 production, it contributes to caspase-11-dependent cell death. These observations demonstrate that caspase-11 plays an important role in responding to bacterial secretion systems that translocate bacterial products into the host cytosol. NAIP5/NLRC4/caspase-1-dependent pyroptosis in response to flagellin plays an important role in restricting *Legionella* replication both in vivo and in vitro. However, whether caspase-11-dependent cell death can also perform this function is not yet known.

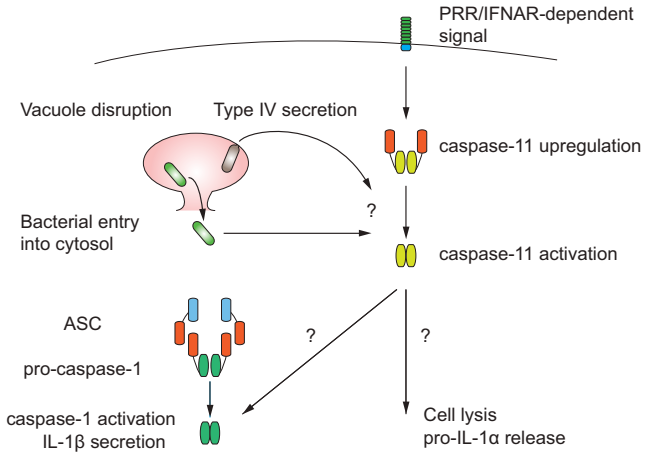

Figure 1. Caspase-11 Activation Detects Bacteria that Access the Host Cytosol

Either activity of type IV secretion systems, as in the case of *Legionella*, or cytosolic entry of bacteria after vacuolar lysis, as in the case of *Burkholderia* or vacuolar pathogens that fail to maintain vacuolar integrity, will trigger caspase-11 activation and caspase-11-dependent cell death. This death is accompanied by release of IL-1α and is independent of canonical inflammasome adapters, sensors, or caspase-1 itself.

Aachoui et al. make similarly elegant use of bacterial and host genetic tools to reveal that bacterial pathogens that enter the cytosol also trigger an ASC/caspase-1-independent but caspase-11-dependent cell death that requires TRIF signaling. This response may therefore also involve the TRIF-IFNAR axis, although the contribution of type I IFN was not specifically investigated. Aachoui et al. observed that although secretion of IL-1β in response to two different pathogenic species of *Burkholderia* required ASC and a combination of NLRP3 and NLRC4, cytotoxicity in response to *Burkholderia* was unaffected, except in *Casp1*$^{-/-}$*Casp11*$^{-/-}$ cells. Using a combination of mouse knockouts, small interfering RNA gene silencing, and retroviral reconstitution, the authors demonstrate that this cytotoxicity requires caspase-11 and not caspase-1. Unlike *Legionella*, *Burkholderia* escapes the phagosome and replicates in the cytoplasm. To test the possibility that cytosolic entry of bacterial pathogens is also a trigger for caspase-11 activation, Aachoui et al. investigated responses to two normally vacuolar pathogens, *Legionella* and *Salmonella typhimurium*, that respectively lacked SdhA and SifA, secreted effector proteins that maintain vacuolar integrity, and thus prevent bacterial mislocalization to the cytosol. Consistent with their observations with *Burkholderia*, infection with Δ*sdhA Legionella* or Δ*sifA Salmonella* led to rapid caspase-11-dependent cell death. Interestingly, in these experiments, Δ*flaA Legionella* did not trigger cell death, unlike the Δ*flaA*Δ*sdhA* mutant; this is in contrast to the Case et al. study, which observed robust caspase-11-dependent cell death in Δ*flaA Legionella*-infected cells. However, this distinction may be due to differences in conditions used to prime the macrophages, as primed and unprimed macrophages respond differently to stimuli that activate different inflammasomes. Caspase-11 was critical for protection against *Burkholderia* infection in vivo, although whether caspase-11-dependent cell death or/and release of caspase-11-dependent proinflammatory signals is responsible for this protection is not yet known.

Together, these two studies reveal that caspase-11 plays a key role in the induction of cell death in response to a functional *Legionella* T4SS in one case, and the entry of pathogens into the cytosol in the other case (Figure 1). The bacterial signal required for caspase-11 activation and caspase-11-dependent cell death remains unknown. In the case of the *Legionella* T4SS, this recognition may be direct, analogous to the recognition of flagellin and structural components of the type III secretion system by the NAIP/NLRC4 system. Alternatively, another bacterial product delivered into the cytosol by the T4SS may be the trigger, and this product may also be released by bacterial pathogens that enter the cytoplasm. Cytosolic bacterial pathogens trigger inflammasome activation through AIM2, which contains a DNA binding domain and a pyrin domain that allows it to interact with ASC and recruit caspase-1. Previous studies demonstrated that SdhA-deficient *Legionella* trigger the AIM2 inflammasome (Ge et al., 2012). However, Aachoui et al. find that ASC is not required for cytotoxicity in response to cytosolic *Legionella*, suggesting that like the NLRP3/ASC inflammasome, the AIM2/ASC inflammasome is required for caspase-1 processing and IL-1β secretion, but not for caspase-11-mediated cell death in response to cytosolic bacteria.

Both the T4SS of *Legionella* and vacuolar disruption by cytosolic bacteria trigger some disturbance of the vacuolar membrane. Thus, caspase-11 could potentially be triggered not by direct cytosolic sensing of bacterial products, but through sensing of vacuolar membrane homeostasis. Vacuolar disruption even in the absence of microbial infection induces NLRP3 inflammasome activation (Hornung et al., 2008). Whether caspase-11 is also triggered under these conditions is unknown. The nature of the upstream sensor that triggers

caspase-11-mediated cytotoxicity is also currently unknown. While TLR4-TRIF-IFNAR signaling upregulates caspase-11 expression and induces its processing, treatment with LPS or type I IFN alone does not trigger caspase-11-dependent cytotoxicity. An additional sensor, which might itself be type I IFN dependent or independent, that responds to specific features of bacterial infection might also be required to induce caspase-11-dependent cell death.

Intriguingly, while caspase-1 can mediate cell death downstream of canonical inflammasomes, caspase-1 is dispensable for caspase-11-dependent cell death. Whether caspase-1 and caspase-11 trigger cell death in the same way therefore remains an open question, and indeed, precisely how caspase-1 or caspase-11 induces cell death is also enigmatic. Together, these new studies shed light on additional signals that activate caspase-11 during bacterial infection and establish caspase-11-dependent cell death as an important effector mechanism for responding to bacterial pathogens that access the host cell cytosol.

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Leishmania Dices Away Cholesterol for Survival

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Host lipid alterations are centrally involved in *Leishmania donovani* infection, and infected patients exhibit hypocholesterolemia. In this issue of *Cell Host & Microbe*, Ghosh et al. (2013) show that the metalloprotease GP63 released by *L. donovani* in the liver cleaves DICER1, inhibiting miR-122 maturation, which regulates cholesterol metabolism. These events decrease serum cholesterol and promote parasite growth.

Protozoan parasites of the genus *Leishmania* are responsible for a spectrum of human diseases, ranging from self-healing ulcers to potentially fatal visceral leishmaniasis (VL), which affect millions of people worldwide. The parasites are transmitted to mammals in the promastigote form by infected sand flies. Following phagocytosis by macrophages, promastigotes differentiate into amastigotes, the mammalian stage of the parasite, and replicate inside phagolysosomal compartments. *Leishmania donovani*, the causative agent of VL, disseminates and multiplies within mononuclear phagocytes of the reticuloendothelial system, including the liver, spleen, lymph nodes, and bone marrow. VL can remain asymptomatic or subclinical or may take an acute or chronic course. Heavily infected patients may develop disease after an incubation period varying from weeks to months. Fever and hepatosplenomegaly are common symptoms. In addition, VL patients suffer from progressive weight loss, cachexia, and pancytopenia. Recent clinical evidence pointed toward an alteration of lipid metabolism in VL patients, characterized by a marked hypocholesterolemia and reduced LDL cholesterol (Lal et al., 2007).

In this issue, Ghosh and colleagues (Ghosh et al., 2013) unveil the mechanism(s) responsible for alteration of cholesterol metabolism in experimental VL. Cholesterol is an essential lipid for the life of eukaryotic cells. It is a major component of biomembranes, as it regulates their fluidity and structural stability and maintenance. Cholesterol plays central roles in processes ranging from signal transduction to hormone synthesis (Simons and Ikonen, 2000). In addition, it is involved in inflammation and infectious disease. Cholesterol biogenesis occurs in the cytoplasm of liver and intestinal cells. Its synthesis starts with the condensation of three acetate molecules to hydroxymethylglutarate by the action of the rate-limiting enzyme hydroxymethylglutaryl-coenzyme A (HMGCoA) reductase. Hydroxymethylglutarate is then reduced to mevalonate, which in turn is decarboxylated to a 5-carbon isoprenoid.